In the Office Action, the Examiner is contending that the specification fails to provide an enabling disclosure of the use of the claimed material in gene transfection based on a statement regarding the primary problem of ex vivo gene therapy is not that the implanted cells fail to remain "in situ" long enough to provide a therapeutic effect, but rather that the level of transgene expression is too low, too unstable and too short lived to provide a therapeutic effect.

Claims 50 and 51 have been rewritten as new claims 80 and 81, which are dependent on claims 58 and 80 respectively. The objective of these claims is to protect the application of the claimed hyaluronic based matrices in cultures of transfected cells to be used in gene therapy. The applicant has developed data which shows that the HYAFF scaffolds when implanted in an animal body retain their morpology three weeks after implantation and become vascularized with new vessels originating from the surrounding vessels. After three weeks, there is clear evidence that the HYAFF scaffolds were undergoing degradation and resorption. The implanted reconstructed tissues comprise skin adnexa, Langerhans islets and hepatocytes.

The data was developed from the following in vivo studies:

Skin Adnexa, cultured for 1 week in a co-culture with human fibroblasts, which were seeded on a non-woven Hyaff® matrix 3 weeks before the skin adnexa, were implanted subcutaneously in nude mice. the animals were divided in three groups each composed of 3 animals and all were implanted in the dorsal region with 1 x 1 cm non-woven Hyaff® matrix prepared as described above, in which, skin adnexa and fibroblasts were present.

The three animals of the first group were sacrificed 1 week after implantation, and the skin of the dorsal region in which the non-woven Hyaff® pieces were present was removed for histological analyses. The pieces were formalin fixed and stained for routine histological observations with hematoxylin and eosin.

The other two groups were sacrificed respectively 2 and 3 weeks after surgery and the skin containing the implanted material was removed for histological analyses as previously described.

The same procedure of implantation was adopted for the pancreatic Langerhans islets and hepatocytes.

The non-woven Hyaff® pieces containing the islets co-cultured with human fibroblasts, seeded 1 week before the pancreatic cells,were implanted in the dorsal region of nude mice. The three groups each composed of 3 animals were sacrificed respectively 1,2 and 3 weeks after surgery.

The dorsal skin was removed as described above. The non-woven Hyaff® pieces containing hepatocytes co-cultured with human fibroblasts, which were seeded 1 week before the liver cells, were implanted by the same procedure described above. The histological observations demonstrated that all three implanted reconstructed tissues, comprising skin adnexa Langerhans.

The Applicants are prepared to provide the above described data in the form of a Rule 132 Declaration if the Examiner deems that to be necessary.

The Examiner has questioned the present specification as not disclosing the efficacy of skin adnexa to produce hair follicles as well as the method of use of the cells for insulin production. This is not within the scope of the present invention but is known in the art. For this reason, the claims are not directed to a therapeutic method but to the novel biological material which provides for a high proliferative rate and long survival times for the cells which provide for more effective results when the cells are transplanted. For these reasons, it is requested that this ground of rejection be withdrawn.

Claims 28-56 were rejected under 35 U.S.C.§112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention.

Reconsideration is requested.

Claims 28-56 have been canceled and new claims 58-86 have been added to point out the invention. Where appropriate, the term "The" has been used in front of --biological material--. The term "sulfated in claims 58 and 59 has been modified to recite "O-sulfated" in accordance with the specification at page 6, lines 2-3 where WO/95/25751 was referenced. That document describes the sulfated derivatives of hyaluronic acid and of hyaluronic acid esters in which the alcoholic hydroxyls present in the polymeric chain of the polysaccharide are sulfated. For this reason, these materials can be described as O-sulfated hyaluronic acid and derivatives thereof as being distinguished from the corresponding NM-sulfated products where the sulfation reaction takes place on the amino groups of the glucosamine portions of the repeating unit of the polysaccharide chain. The term "heterocyclic" has been inserted into new claims 58 and 59. For these reasons, it is requested that this ground of rejection be withdrawn.

Claims 29-32, 35-43, 49, 52, 53, and 56 were rejected under 35 U.S.C.§103(a) as being unpatentable over Soranzo et al. in view of Cialdi et al. and Dorigatti et al.

Reconsideration is requested.

The Soranzo reference notes at page 5, lines 5-10 that the purpose of their invention is provide artificial skin which simulates the epidermal and the dermal layer of natural skin wherein both fibroblasts and keratinocytes are present. The artificial skin is formed by an upper microperforated membrane which is a two dimensional matrix based on a hyaluronic acid derivative. The keratinocytes are seeded and left to proliferate on the microperforated membrane which is on top of a non-woven tissue (i.e. a three-dimensional matrix) which is based on a hyaluronic acid derivative which has been seeded with fibroblasts that are left to proliferate. The two layers are separated at the interface by the protein extracellular matrix which has the characteristics of a dermoepidermal junction.

The biological material of the present invention has at least one cell type selected from endothelial cells,

glandular cells, skin adnexa, germinative cells of hair bulbs and optionally keratinocytes and a three dimensional matrix comprising at least one hyaluronic acid derivative and optionally collagen and/or fibrin. The artificial skin disclosed by Soranzo et al., besides beings completely different from the present biological material, does not suggest the present invention.

As clearly stated in the present application at page 2, lines 13-28, weak and fragile differential cells such as endothelial, glandular cells, islet of Langerhans, liver cells or skin adnexa, are more difficult to isolate and culture onto artificial or plastic supports than other type of cells like stem cells, fibroblasts, keratinocytes, etc. In addition they show poor proliferative properties and short survival times.

For example, liver cells can survive in vitro for about 7 weeks, with less than 50% of the cells remaining viable. Skin adnexa cells last about 2 weeks, and islet of Langerhans just a few days. It therefore follows that, although the properties of hyaluronic acid derivatives (and in particular the hyaluronic acid esters namely the HYAFF matrices are already known to favor the growth and the development in vitro of resistant and very active cellular elements such as stem cells or fibroblasts, etc., one who is skilled in this art would not have been able to predict that satisfactory proliferation rates and survival times can be achieved by cultivating the aforementioned weak and fragile cells on supports made of hyaluronic acid derivatives.

The Applicants have surprisingly found that also poorly resistant and weak cells such as endothelial cells, glandular cells and adnexa, germinative cells of hair bulbs, etc. can be efficiently grown on a hyaluronic acid derivative matrix.

Soranzo et al. as highlighted above, disclose an artificial skin which does not contain the above mentioned weak and fragile cells, and could therefore not suggest to a skilled person that it was possible to overcome the technical problem pointed out above, with the present three dimensional

supports made of a hyaluronic acid ester.

Dorigatti et al. refer to a non woven fabric material comprising fibers of at least one hyaluronic acid ester, alone or in combination with fibers of another polymer. This reference is cited in the present application, page 6, line 6 wherein, the Applicants noted that the three-dimensional matrix in the biological material according to the present invention may be constituted by the non-woven fabric material disclosed by Dorigatti et al. In addition, it is worthy to note that this reference does not mention the presence of cellular components.

The teachings of Soranzo et al., even in combination with Dorigatti et al., do not in any way suggest to the skilled person in the art that it was possible to overcome the problems associated with the weak and fragile cells like those present in the biological material as presently claimed, i.e. endothelial cells, glandular cells, skin adnexa, and germinative cells of hair bulbs, by cultivating and growing such cells on a three-dimensional support containing a hyaluronic acid derivative, such as that disclosed by Dorigatti et al.

The biological material as claimed in claim 59, which comprises the above said cells cultivated in presence of a medium treated with fibroblasts or in a culture with fibroblasts, is not suggested by Soranzo et al., who teach an artificial skin which must always contain both keratinocytes and fibroblasts, without any of the present cell types, i.e. endothelial cells, glandular cells, skin adnexa, and germinative cells of hair bulbs.

As regards to Cialdi et al., we note that this reference relates to sulfated hyaluronic acid and derivatives thereof, which possess anticoagulant properties and can be employed for the preparation of biomaterials which come into contact with blood or highly vascularized tissues. As correctly noted by the Examiner, Example 14 of Cialdi et al. discloses the co-culturing of human umbilical vein endothelial cells in the presence of sulfated hyaluronic acid, which

results in the formation of a confluent monolayer in a shorter time than a control which is made of un-sulfated hyaluronic acid. According to Example 15 of Cialdi et al, this sulfated hyaluronic acid functions also as a heparin-like product in inducing angiogenesis and neovascularization in vitro.

The PCT Application WO 95/25751 corresponding to the US Patent indicated by the Examiner as Cialdi et al. which was cited in the present application, page 6, line 2 wherein the Applicant affirms that the sulfated hyaluronic acid and derivatives thereof therein described may be used for the preparation of the present matrix.

The Applicants wish to point out that this reference does not any way suggest, alone or in combination with the others cited documents, the invention as presently claimed for the following reasons:

1) It is essential for the biological material according to the present invention that the support which is made of hyaluronic acid must have a three-dimensional structure, whereas Cialdi et al. does not deal with the structure of the sulfated hyaluronic acid. The growth of endothelial cells described in Example 14 is carried out in the presence of sulfated hyaluronic acid as a culture additive; the control used in this Example is in fact un-sulfated hyaluronic acid.

2) The sulfated hyaluronic acid derivatives may be used for the preparation of the biological material according to the present invention, as well as many other classes of hyaluronic acid derivatives which are different from the sulfated derivatives, may be successfully used for growing poor resistant and weak cells, such as endothelial cells, thanks to the fact that the hyaluronic acid derivatives are processed in the form of a three-dimensional matrix.

Cialdi et al. do not deal with a biological material having a three-dimensional support based on a hyaluronic acid derivative, but only with a culture substrate, from which presumably the cells separate once they reach maturation. These cells cannot be used as a scaffold or as a support for the same cells. On the contrary, in the present biological

material, the three-dimensional support consisting of hyaluronic acid derivatives is used as scaffold for the cells, from which the cells do not separate even after reaching maturation.

In view of the foregoing, Cialdi et al. also in combination with Soranzo et al. do not in any way suggest the presently claimed invention, since Soranzo et al. disclose an artificial skin, that does not contain the above mentioned weak and fragile cells and fails to suggest to the skilled person in the art that it was possible to overcome the technical problem pointed out in the present application by using the three-dimensional supports made of hyaluronic acid derivatives. Cialdi et al. is silent about this essential characteristic of the instant biological material, namely that the support made of hyaluronic acid derivative must have a three-dimensional structure.

The objections to Figs. 5-9 has been noted. Formal drawings will be provided upon the indication of allowable subject matter.

The Examiner is thanked for indicating that claims 33, 34, 54 and 55 for a biological material are free of the prior art. These claims have been represented as claims 62, 63, 83 and 84.

An early and favorable action is earnestly solicited.

Respectfully submitted,

James V. Costigan
Reg. No. 25,669

MAILING ADDRESS:
HEDMAN, GIBSON & COSTIGAN, P.C.
1185 Avenue of the Americas
New York, NY 10036-2601
(212) 302-8989

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on 10/23/08